REVIEW

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Reciprocal interactions of the intestinal microbiota and immune system

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The emergence of the adaptive immune system in vertebrates set the stage for evolution of an advanced symbiotic relationship with the intestinal microbiota. The defining features of specificity and memory that characterize adaptive immunity have afforded vertebrates the mechanisms for efficiently tailoring immune responses to diverse types of microbes, whether to promote mutualism or host defence. These same attributes can put the host at risk of immune-mediated diseases that are increasingly linked to the intestinal microbiota. Understanding how the adaptive immune system copes with the remarkable number and diversity of microbes that colonize the digestive tract, and how the system integrates with more primitive innate immune mechanisms to maintain immune homeostasis, holds considerable promise for new approaches to modulate immune networks to treat and prevent disease.

Every one of us enters the world devoid of microbial colonization because of the sterile environment of the womb. This germ-free existence is short-lived: birth exposes the newborn to the microbiota of the mother, setting in motion the colonization of mucosal tissues in the digestive, respiratory and urogenital tracts, and the skin by a diverse microbiota, which we coexist with throughout our lives. The complex and dynamic interaction between the microbiota and its human host is the culmination of nearly half a billion years of co-evolution with vertebrates that has reciprocally shaped the repertoires of the microbiota and the immune system, such that the microbiota in humans is normally restrained and well-tolerated. The scope of this interaction is particularly evident in the intestinal tract, in which the greatest diversity and abundance of microbes reside. Estimated at approximately 100 trillion organisms, most of which are bacteria (although archaea and eukaryotes are also represented), the microbiota numbers about ten times the total cells in the human body, with the greatest density populating the distal ileum and colon[1]. The collective genome, or metagenome, of the intestinal microbiota has more than 100 times the number of genes of the human genome. Each individual is populated by roughly 15% of the 1,000 or more species of intestinal bacteria that have been described[2], which reflects the substantial variability in the composition of the microbiota between individuals. There are, therefore, about tenfold more genes in each of our microbiomes than in each of us, encoding the greatest source of potential antigens for the immune system to cope with, substantially exceeding those of self and pathogen-derived antigens.

The relationship that has been forged between the intestinal microbiota and its human host provides mutual benefits. At homeostasis, the microbiota benefits from the warm, nutrient-rich environment of the gut so it can establish a relatively stable ecosystem. Humans in turn benefit from a highly adaptive metabolic engine that in addition to providing essential non-nutrient factors, such as vitamins, also substantially increases our ability to harvest nutrients from food. This increased digestive capacity is mainly a result of the microbiota's complementation of the limited diversity of complex-carbohydrate-metabolizing enzymes that are encoded in the human genome. In addition, by establishing robust, interlinked metabolic or nutrient networks, and biofilms among its constituents, the microbiota limits the resources available to potential pathogens that must outcompete well-adapted and entrenched resident microbes for metabolic and physical niche space. Resident microbes thereby establish a microbial buffer that limits access by those not part of the consortium.

However, the microbiota is not innocuous, and under conditions that compromise the ability of the host to limit the microbiota's entry from the intestinal lumen, some species can invade host tissues to cause disease. Furthermore, shifts in the composition of the microbiota, whether induced by dietary changes, antibiotic treatment or invasive pathogens, can disturb the balance of organisms in the microbiota and alter the metabolic network of the collective to favour the outgrowth of potentially pathogenic constituents. Referred to as dysbiosis, such changes in the microbiota can perturb immune regulatory networks that normally restrain intestinal inflammation, and may contribute to immune-mediated disease directed against antigens of the microbiota. Dysbiosis is most often associated with inflammatory bowel disease (IBD), including Crohn's disease and ulcerative colitis, and necrotizing enterocolitis in premature infants. But it is also increasingly linked to a number of extraintestinal immune-mediated diseases, including rheumatoid arthritis, multiple sclerosis, diabetes, atopic dermatitis and asthma, as well as obesity and metabolic syndrome, all of which could have their pathogenic origins in untoward reactivity of the immune system to the microbiota. Whether dysbiosis is a cause or effect of these disorders remains to be determined, but understanding the factors that lead to alterations in the composition of the microbiota in these conditions promises to be informative, irrespective of their basis.

In this Review, we highlight advances in our understanding of the immune mechanisms by which the dynamic interplay of the intestinal microbiota and its host normally favours a homeostatic, mutualistic relationship, as a basis for understanding the causes of breakdowns in this relationship that lead to disease.

Co-evolution of the microbiota and adaptive immunity

Vertebrate evolution coincided with the emergence of the adaptive immune system, the main components of which are T and B lymphocytes. The acquisition of genetic recombinatorial mechanisms for generating diverse, anticipatory antigen-recognition receptors on T cells and B cells allowed specific responses to a vast diversity of antigens, and long-lived immune memory. Although evolutionary pressures that have shaped immune strategies are often viewed in the context of host defence, it has been proposed that the emergence of adaptive immunity

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could have been driven as a means to foster, rather than limit, microbial colonization[3]. In this view, a substantial survival advantage would have been afforded to organisms that could harness the extended and more flexible metabolic capacity derived from a permanent, diverse intestinal microbiota, as long as the attendant infectious risk could be mitigated. Adaptive immunity might have allowed this.

By adding new layers — such as secretory IgA (sIgA) — to existing innate barrier defences, early vertebrates with adaptive immune mechanisms could begin to promote colonization of their alimentary tracts by exerting selective pressures that favoured resident microbes that were relatively innocuous, but metabolically useful. The survival benefits that accrued as a result of derivation of nutrients from a broader range of foods could have been a major factor in the evolutionary success of vertebrates[3]. The emergence of adaptive immunity provided a means to recognize, and remember, both beneficial and detrimental members of the intestinal microbiota to foster maintenance of beneficial species at the expense of detrimental ones. In doing so, the emerging adaptive immune system would have had to acquire mechanisms for tempering innate immune responses programmed only for the clearance of microbes. The development of a broad repertoire of immune cells that could suppress, as well as promote, innate inflammatory mechanisms contingent on the threat level of the microbe they recognized would become invaluable.

Support for this hypothesis comes from the study of the diversity of the microbiota in invertebrates, which seems to be far less complex than the microbiota of vertebrates[3]. Although data on comparative metagenomics are limited, the highest density of bacteria found in either organisms or the environment is in the human colon[1]. The intestinal microbiota of vertebrate species as divergent as rodents and zebrafish share similar complexity to that of humans, and their gastrointestinal tracts are similarly colonized soon after birth[4]. Further, a homologue of sIgA was recently identified in bony fish[5]. Now extending to all jawed vertebrate taxa, except cartilaginous fish and reptiles, this unique class of immunoglobulin is adapted for interactions with the commensal microbiota and mucosal pathogens. It is tempting to speculate that the development of multimeric mucosal IgA, and adaptations that effect its efficient transport across the mucosal epithelium, and the compartmentalization of the gut-associated lymphoid tissues (GALT) from the peripheral immune system were essential adaptations that allowed vertebrates to harness a complex intestinal microbiota. Although more work is needed to establish a comparative map of the microbiota in the digestive tracts of more vertebrate and non-vertebrate species, it is anticipated that the merger of comparative studies in metagenomics and immunology will yield insight into the adaptive mechanisms that have allowed vertebrates to become an evolutionary success. It may yet prove that we owe it to the bugs that have become 'us.'

The microbiota and the developing immune system

The mammalian immune system is perhaps the most elaborate example of the complex symbiotic relationship that has resulted from the co-evolution of vertebrates and their microbiota. Unlike other vertebrates, placental mammals give birth to live young that are carried to term *in utero* and are nursed with milk that is rich in maternal antibodies. These antibodies provide passive transfer of immunity from mother to infant, which has implications for the developing immune system of the infant, and the microbiota that colonize the gut.

Maternal effects on the neonatal microbiome

The greatest initial contribution to the composite human–microbiota 'superorganism' is the vertical transmission of components of the mother's microbiota to the child at birth. Normally, intestinal colonization of neonates is dominated by transmission of bacteria from the maternal vaginal flora, which is less diverse than that of the lower intestinal tract[6]. The 'pioneer' species received from the mother seem to be important, because infants born by Caesarean section — who are initially colonized by bacterial species of epidermal, rather than vaginal, origin — are predisposed to development of allergies and asthma later in life[7]. This is despite the fact that, although Caesarean-section-born infants lag behind those born transvaginally in their acquisition of the two bacterial divisions dominant in the adult microbiota (Firmicutes and Bacteroidetes), they do catch up. Conversely, mother-to-child transmission in neonates delivered transvaginally rapidly shifts to skin and oral ecologies postpartum, such that these infants are later exposed to similar species as those delivered through Caesarean section. Thus, first contact could be deterministic. That is, pioneer bacterial species might have substantial and lasting effects on the immune response, irrespective of the composition of the mature microbiota.

The neonatal microbiota varies erratically until about 1-year-old when it stabilizes, establishing a consortium that resembles that of adults[8]. During this initial period, the neonatal immune system rapidly matures under the influence of the microbiota. Although environmental factors such as diet, exposure to new microbes, xenobiotics, bacteriophages and intestinal infections have important roles in shaping the composition of the microbiota during this maturational window, the part the neonatal immune system plays is less clear. What is clear is the initial and ongoing influence of breastfeeding on the infant's microbiome. In addition to a unique mix of nutrients and antimicrobial proteins that influence the ecology of the neonatal microbiota, breast milk provides abundant sIgA, the specificities of which have been shaped by the maternal microbiota. The mucosal immune memory of the mother is thereby transmitted to her offspring. Thus, in breastfed infants delivered transvaginally, the intestinal microbiota is not only seeded by maternal bacterial species, but its composition may also be reinforced and shaped by the maternal sIgA repertoire that is influenced, in turn, by the maternal microbiota.

Maturation of the infant mucosal immune system takes months, so the passive transfer of maternal sIgA has a considerable protective role against potential pathogens that could perturb the ecological trajectory of the infant's intestinal microbiota. Maternal sIgA also shields the neonatal immune system from its own microbiota, perhaps so the neonate's defences are not overwhelmed before they are fully developed. In so far as microbial antigens that are bound by sIgA are handled by the innate immune system in a 'tolerogenic' mode, partly owing to IgA's poor fixation of complement, transfer of maternal sIgA to the infant seems to favour the establishment of regulatory immune networks in the infant that promote a mutualistic relationship with the microbiota. Thus, in addition to the direct effect of maternal sIgA on the microbiota of the infant, it also has immunomodulatory effects on the developing infant's immune repertoire so as to indirectly influence its microbiota. The maternal–neonate metagenomic bond is, therefore, extended postnatally, providing one mechanism by which microbial ecologies tend to cluster in family members.

Microbiome effects on developing host–barrier defence

Maturation of the intestinal mucosa and its GALT — Peyer's patches of the distal ileum, isolated lymphoid follicles (ILFs) and mesenteric lymph nodes (MLNs) — is initiated by, and contingent on, intestinal colonization. Peyer's patches and MLNs develop prenatally, but ILFs develop postnatally[9]. However, each of these lymphoid tissues requires signals derived from the sensing of intestinal microbiota for their complete development, recruitment of a mature complement of immune cells, or both. Similarly, non-lymphoid structures of the intestinal mucosa that contribute to the establishment of host–microbiota mutualism are driven by colonization of the neonate.

Preparation for the adaptive immune response to neonatal colonization requires the prenatal actions of a subset of innate lymphoid cells (ILCs), termed lymphoid tissue inducer (LTi) cells (Fig. 1). LTi cells are instrumental in prenatal organization of the development of lymphoid tissues, including the components of the GALT. LTi cells develop in the fetal liver from a common lymphoid precursor that gives rise to all lymphoid cells. During fetal development, LTi cells disseminate to the MLN and Peyer's patches anlagen, stimulating the development of these structures, and the recruitment and partitioning of B and T cells into B-cell follicles and T-cell zones that characterize secondary lymphoid tissues[9,10].

The development of ILFs is also dependent on LTi cells, but is initiated

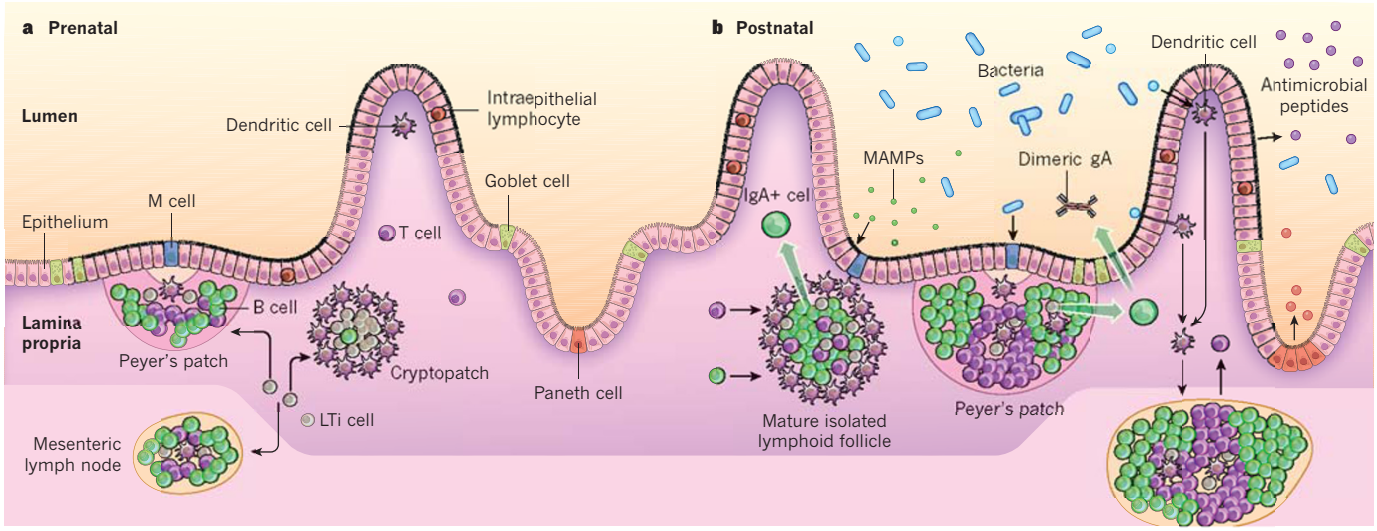

Figure 1 | The gut-associated lymphoid tissue establishes perinatal host-microbiota mutualism in the intestine. **a**, Prenatally, secondary lymphoid tissues (Peyer's patches and mesenteric lymph nodes) and cryptopatches develop by the spatiotemporal recruitment of lymphoid tissue inducer (LTi) cells to sites of the developing intestine and supporting neurovascular structures. This, in turn, stimulates the recruitment of dendritic cells, T cells and B cells in preparation for the immune response to the microbiota. Intraepithelial lymphocytes seed the epithelium before birth. **b**, Postnatally, bacteria colonize the neonatal intestine immediately, initiating multiple events that affect the development or functional maturation of the mucosa and gut-associated lymphoid tissues. Shown from left to right: microbe-associated molecular patterns (MAMPs) sensed by pattern-recognition receptors on intestinal epithelial cells and dendritic cells adjacent to cryptopatches stimulate the further recruitment of B cells and T cells, causing the cryptopatches to develop into mature isolated lymphoid follicles. The isolated lymphoid follicles release IgA-producing plasma cells — which are formed through T-cell-dependent and independent interactions — into the lamina propria. Microbes also cross the epithelium and enter Peyer's patches through M cells, from which they are endocytosed by dendritic cells in the subepithelial dome. Antigen-loaded dendritic cells in the Peyer's patch interact with local lymphocytes to induce T-cell differentiation and T-cell-dependent B-cell maturation in the germinal centre to induce the development of IgA-producing plasma cells that home to the lamina propria, where they release dimeric IgA for transport into the intestinal lumen. Dendritic-cell-mediated luminal sampling of microbial products or transcytosis of bacteria across the epithelium results in antigen loading of lamina propria dendritic cells, which then migrate through the afferent lymphatics vessels (not shown) to a draining mesenteric lymph node to induce differentiation of effector T cells that traffic to the lamina propria. Shown on the far right, sensing of MAMPs stimulates the proliferation of intestinal epithelial cells in crypts, resulting in their increased depth and, in the small intestine, increased density of Paneth cells. This sensing also arms the intestinal epithelial cells for release of antimicrobial peptides.

after birth when these cells cluster in the lamina propria below the intestinal crypts, forming cryptopatches[11]. ILFs develop from cryptopatches only after colonization by the intestinal microbiota. Central to sensing the colonizers of the intestinal tract is the expression of a diverse range of germline-encoded pattern-recognition receptors (PRRs) by intestinal epithelial cells (IECs) and immune cells resident in the gut. These receptors include transmembrane Toll-like receptors (TLRs) and C-type lectin receptors (CLRs) that reside on the cell surface or in endosomes, and cytosolic nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), which cells of the intestinal mucosa use to detect microbe-associated molecular patterns (MAMPs) that are expressed by constituents of the resident microbiota, as well as pathogens. MAMPs are recognized — through mechanisms that are not well understood — by cells in the neonatal gut, stimulating ILF development to generate a lymphoid structure that is capable of supporting the maturation of B cells that produce sIgA. At least one of the microbial products that induces this process is peptidoglycan derived from Gram-negative bacteria. Recognition of peptidoglycan by NOD1 in IECs elicits production of CCL20 and β-defensin 3 that direct the recruitment of B cells to LTi–dendritic-cell clusters in cryptopatches to induce the expression of sIgA (ref.12).

Unlike MLNs and Peyer's patches, for which class-switch recombination to produce IgA is T-cell dependent, IgA class-switching in ILFs can occur through T-cell-dependent and -independent pathways[13]. In addition, MLNs and Peyer's patches are unique to mammals, whereas ILFs are found in non-mammalian vertebrates, suggesting that they are the evolutionary forerunners of the secondary lymphoid tissues of the GALT[14] and may have evolved to accommodate, and promote, an increasingly complex intestinal microbiota before specialized secondary lymphoid tissues emerged. The importance of ILFs in regulating the microbiota is evident in mice that are devoid of these structures, and in which Gram-negative bacteria are over-represented[12]. Similar to Peyer's patches, ILFs develop in the intestinal mucosa in intimate association with the overlying intestinal epithelium (Fig. 1). Both structures are strategically deployed to sample the microbiota through specialized microfold, or M, cells, which transport intact microbes or their products across the epithelial barrier. In this way, the innate and adaptive immune systems constantly monitor the microbiota and are 'informed' of the dominant bacterial species that reside in proximity to the epithelium.

Like the GALT, the epithelium of the fetal and newborns' intestine is incompletely adapted to dense bacterial colonization, but responds rapidly to perinatal colonization to accommodate the microbiota. Expression of — and signalling by — TLR4, which is the receptor for the abundant product of Gram-negative bacteria, lipopolysaccharide, are significantly increased antecedent to intestinal colonization at birth[15]. Within hours of exposure to the microbiota, the response of IECs to lipopolysaccharide is markedly attenuated through the down-regulation of TLR4 and components of its signalling apparatus. This increase in TLR4 expression and signalling followed by down-regulation seems to represent a mechanism whereby the fetal epithelium is primed to transmit transient inflammatory signals on sensing the microbiota to promote an early immune response, but then becomes rapidly desensitized as an adaptation to the mounting bacterial load.

An additional immune mechanism that prepares the neonate for commensal colonization is the seeding of the intestinal epithelium with specialized intraepithelial lymphocytes before birth. Intraepithelial lymphocytes intercalate between IECs where they exist in an activated state and can respond rapidly to microbial encroachment. Throughout life, intestinal intraepithelial lymphocytes help to maintain the integrity of the epithelial-cell barrier, limit bacterial translocation and facilitate epithelium repair after injury by secreting soluble mediators, such as antimicrobial peptides[16].

Although the host has evolved elaborate developmental strategies to prepare for postnatal colonization, the microbiota reciprocates by inducing maturation of the host's immune system. Therefore, compared with conventionally raised mice, germ-free or 'germ-reduced' mice have reduced size and cellularity of secondary lymphoid tissues as well as altered numbers, frequencies or diversity of immune-cell populations at mucosal sites, and thus mount abnormal responses to infection and injury. In addition, a postnatal time window is thought to exist in which regulatory T (T_{reg}) cells[17] and invariant-natural killer T (iNK T) cells[18] that have received their 'primary education' in the thymus, receive a 'secondary education' from the microbiota to establish a durable immune repertoire that is crucial to the prevention of inflammatory diseases in childhood and adulthood.

Innate pathways to sense and restrain the microbiota

The intestinal tract is the largest barrier tissue in the human body (it has a surface area of about 300 m^3 in adults), making it the most extensive portal for entry of commensal or pathogenic microbes. Highly specialized barrier defences have evolved to confine the microbiota and resist pathogens, while maintaining its main function of nutrient uptake. The strategy is one of a layered defence that integrates a stratified mucous layer, a relatively impenetrable but highly responsive epithelium, and a lamina propria populated by innate and adaptive immune cells that actively participate in homeostatic responses to restrain the microbiota without undue inflammation, yet are poised for the induction of antimicrobial clearance responses and tissue repair, should the barrier be breached.

Reciprocity between the epithelium and the microbiota

The epithelium is central to the orchestration of intestinal defences. Not simply a passive barrier to microbial translocation, the intestinal epithelium is an active sensor of, and conduit for, the dialogue between the host and microbiota. The five main cell types that comprise the epithelium — absorptive enterocytes, goblet cells, Paneth cells, M cells and enteroendocrine cells — develop from a common stem cell located near the base of the intestinal crypts. Each cell type has a specialized, integral role in intestinal homeostasis, and is both responsive to the microbiota and conditioned by it (Fig. 1).

Maintaining the polarized structure of the epithelial barrier is crucial to its function. The tight junctions that seal the interfaces of adjacent IECs segregate the epithelium into an apical, lumen-exposed surface and a basolateral surface anchored to the basement membrane. Depending on whether the membrane-associated PRRs are arrayed on the apical or basolateral surface of the IECs and on which side of the epithelial barrier MAMPs are detected, cells of the epithelium initiate responses that either promote the release of protective factors that are directed luminally (for example, secreted mucins and antimicrobial peptides) to directly restrain the microbiota, or internally (for example, cytokines and chemokines) to either promote immune quiescence at homeostasis or activate inflammatory immune responses when the epithelial barrier has been breached.

As a mucosal tissue, the intestinal epithelium continuously produces and is invested by a layer of mucus that is a first line of defence against microbes (Fig. 2). Mucus is produced by goblet cells and is composed of heavily glycosylated mucin proteins, as well as other protective molecules, such as trefoil factor, that contribute to epithelial restitution and repair. Production of intestinal mucus is regulated by products of the microbiota. In germ-free mice the mucous layer in the colon is highly attenuated, despite normal numbers of mucin-laden goblet cells. Addition of the MAMP lipopolysaccharide or peptidoglycan stimulates the release of mucin by goblet cells and the rapid reconstitution of the colonic inner mucous layer[19]. Butyrate produced by benign constituents of the microbiota also promotes increased release of mucin, providing a positive-feedback loop for maintenance of the mucous barrier and its colonization by butyrate-producing commensals. The importance of the mucus layer is evident in mice deficient for principal intestinal mucin, Muc2. These mice have increased translocation of commensal and pathogenic bacteria[20], and spontaneously develop colitis[21]. Components of the healthy microbiota therefore directly contribute to the barrier function of the intestine through their induction of mucin production and secretion by goblet cells.

The thickness and continuity of intestinal mucus differs regionally: it is thinner and discontinuous in the proximal small intestine and becomes thicker and continuous in the distal small and large intestine[22], showing some correlation with the local bacterial load (10^3–10^5 organisms per gram of luminal contents in the duodenum and jejunum; about 10^8 organisms per gram in the ileum, and 10^{10}–10^{12} organisms per gram in the colon). The mucus is stratified into two functionally distinct layers: a compact, firmly adherent inner layer that is sparsely populated by bacteria and a more loosely structured, non-adherent outer layer that is at least tenfold more densely populated by the microbiota[22].

Although both mucous layers have a similar Muc2-dominated composition, proteolytic cleavage of Muc2's polypeptide backbone in the outer layer results in its expanded volume and accessibility to colonization by components of the microbiota[22]. Indeed, the outer mucous layer provides an anchor for the attachment of bacteria of the microbiota that can establish biofilms that exclude pathogens. Furthermore, in addition to dietary glycans, mucin glycans are nutrients for some constituents of the microbiota, such as *Bifidobacterium* and *Bacteroides* spp., thereby promoting their retention in the collective (Box 1). These bacteria ferment complex O-linked mucin glycans to produce short-chain fatty acids (SCFAs) derived from mucin catabolism (for example, acetate and lactate) that are toxic to some pathogens[23], and produce other metabolites (such as, proprionate and butyrate) that are the main nutrient source for colonic IECs. The SCFAs also signal through

BOX 1

Modulation of the microbiota by blood-group antigens

An important aspect of the microbiota's use of the intestinal mucous layer as an ecological niche is its decoration by blood-group antigens. Analogous to blood-group antigens on erythrocytes, the assembly of type A, B or Lewis-b glycans on intestinal mucins is contingent on the generation of the core H-glycan by the actions of FUT2. This protein is encoded by *FUT2*, which is functional in most individuals (secretor genotype), but is non-functional in a significant minority (about 20% of Caucasians, referred to as non-secretors) owing to a missense mutation. The presence or absence of a functional *FUT2* allele correlates strongly with the composition of the microbiota[97]. In particular, *Bifidobacterium* spp., which are a beneficial component of the microbiota, are dependent on the terminal blood-group glycans for colonization of the intestinal mucus and are less abundant in non-secretor genotypes. The non-secretor phenotype is associated with necrotizing enterocolitis and Gram-negative sepsis in premature infants[98], as well as those with Crohn's disease[99]. Therefore, it seems that the protective benefits of colonization by *Bifidobacterium* spp. are a mutually beneficial evolutionary adaptation of this commensal to its host. Given the predisposition to dysbiosis and its deleterious health effects in non-secretors, it is likely that evolutionary pressure to retain the defective *FUT2* allele in the population is a result of mucin-linked blood-group glycans also serving as sites of attachment for mucosal viruses (such as noroviruses) so that those with a non-secretor genotype are protected[100].

G-protein-coupled receptors on IECs, to downregulate host inflammatory responses[24]. Thus, colonization of the outer mucous layer by commensals is an important adaptation that supports a stable host–microbiota relationship.

In contrast with the outer mucous layer, the inner mucous layer provides a relatively impermeable barrier against the microbiota. This characteristic is a result of the layer's compact physicochemical structure and its function as a reservoir for microbicidal products of the epithelium, including antimicrobial peptides that are specialized to kill different classes of microbes and sIgA, which is retained in the mucous layer after being shuttled across the epithelium by polymeric immunoglobulin receptor (pIgR) (Fig. 2). Effectively, the inner mucous layer is a 'killing field' that few pathogens or commensals have evolved strategies to penetrate. This microbe-sparse zone is enforced, in part, by the antibacterial lectin Reg-IIIγ (ref. 25). Because Reg-IIIγ is selectively bactericidal for Gram-positive bacteria, it is likely that antimicrobial peptides specific for Gram-negative bacteria also contribute to the microbe-sparse zone. A similar spatial segregation of bacteria from the epithelium has been identified in the colon, in which a thicker inner mucous layer excludes most bacteria even in the face of a substantially higher bacterial load[22].

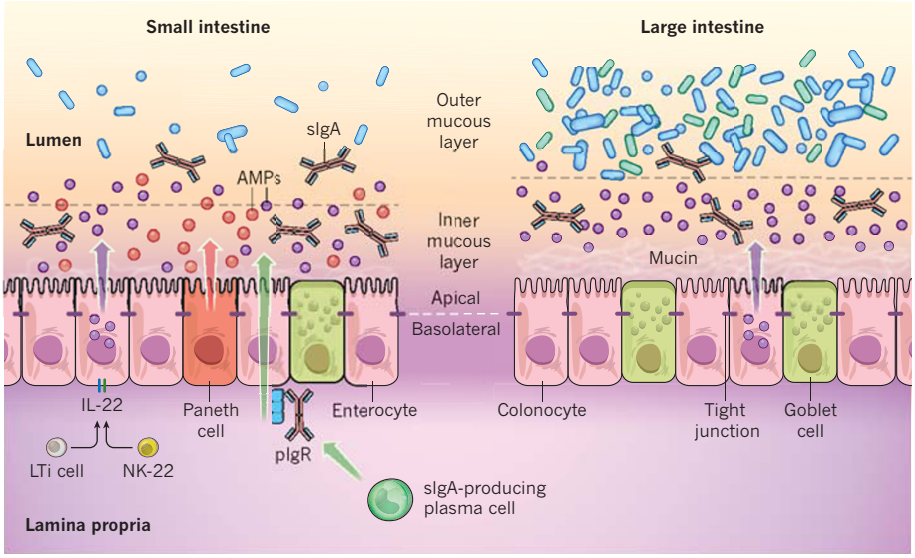

Figure 2 | The barrier function of the intestinal epithelium. Distinct subpopulations of intestinal epithelial cells (IECs) are integrated into a continuous, single cell layer that is divided into apical and basolateral regions by tight junctions. Enterocytes, in the small intestine, and colonocytes in the large intestine, as well as specialized Paneth cells in the bases of small intestinal crypts continually sense the microbiota to induce the production of antimicrobial peptides (AMPs). Goblet cells produce mucin, that is organized into a dense, more highly cross-linked inner proteoglycan gel that forms an IEC-adherent inner mucous layer, and a less densely cross-linked outer mucous layer. The outer layer is highly colonized by constituents of the microbiota. The inner mucous layer is largely impervious to bacterial colonization or penetration due to its high concentration of bactericidal AMPs, as well as commensal-specific secretory IgA (sIgA), which is ferried across IECs from their basolateral surface, where it is bound by the polymeric immunoglobulin receptor (pIgR), to the inner mucous layer where it is released by proteolytic cleavage of pIgR. Responding to the microbiota, innate lymphoid cells, including RORγt- and AhR-expressing LTi and NK-22 cells, produce IL-22, which stimulates AMP production and promotes epithelial barrier integrity.

The region of the intestinal tract with the poorest coverage of protective mucous, the proximal small intestine[22], also has the greatest exposed epithelial surface area owing to its prominent villous structure. Although this feature favours digestion and absorption of nutrients it also seems to make the epithelium in this region particularly vulnerable to entry by the microbiota and pathogens. However, the bacterial loads in this region are the lowest along the length of the intestine, with over a million-fold fewer bacteria per unit of luminal contents than the large intestine. This is, in part, owing to the more vigorous peristaltic motility of the proximal small intestine, which rapidly clears material, including microbes, from the lumen. The lumen of the proximal small intestine is also the entry point for the contents of the gall bladder and stomach, which contain high levels of bile salts and acid that have antimicrobial effects.

In addition, the bases of the crypts of the small intestine are home to many Paneth cells. These IECs are arrayed with a range of PRRs and are specialized for the production and release of abundant antimicrobial peptides, including α-defensins, which are small, highly cationic microbicides unique to Paneth cells. In contrast with other antimicrobial peptides, such as Reg-IIIγ, synthesis of which requires signals from the microbiota[26], α-defensins are synthesized and stored in Paneth-cell granules without the need for sensing of MAMPs. However, the release of Paneth-cell granules is induced by MAMPs, and the secretion of active α-defensins has been shown to control the composition of the microbiota[27]. *NOD2,* which was the first susceptibility gene linked to Crohn's disease[28,29], encodes an NLR that is important in sensing the microbiota to control the release of antimicrobial peptides by Paneth cells. Deficiencies of antimicrobial peptide production, such as occur in NOD2 mutants, are thought to result in altered microbiota composition and density in the small intestine that heighten susceptibility to intestinal inflammation, particularly in the terminal ileum where the highest density of Paneth cells are found.

Colonic IECs also regulate the composition of microbiota through PRR-dependent mechanisms. Mice deficient in the NLRP6 inflammasome in colonic IECs have an altered microbiota that confers increased susceptibility to colitis because of damage to the colonic epithelium[30]. The increased susceptibility to colitis that results from NLRP6 deficiency is transmissible to wild-type mice, indicating that a dysbiotic flora is a contributory factor. How deficiency of the NLRP6 inflammasome results in an altered microbiota is incompletely defined, although reduced interleukin (IL)-18 levels in NLRP6-deficient mice suggests this cytokine has an important role.

Reciprocity between the epithelium and innate immune cells

Although the intestinal mucous layer largely insulates the intestinal epithelium from direct interactions with the microbiota, bacterial metabolites and components are able to permeate this zone and alter gene expression in IECs through PRRs[31]. In addition to the epithelial products that are secreted apically to restrict contact with the microbiota (for example, mucins and antimicrobial peptides), the epithelium also produces factors, such as chemokines and cytokines, that are secreted basolaterally. These factors signal immune cells residing internal of the epithelium, particularly in the intestinal lamina propria (Fig. 3). Activation of PRRs typically promotes pro-inflammatory innate responses, so the intestinal epithelium had to evolve strategies to mitigate these responses for commensals such that, at homeostasis, cytokine signals transmitted to mucosal immune cells limit inflammation. Several mechanisms by which this anti-inflammatory state is favoured have emerged.

The strategic distribution of TLRs and NLRs on and within IECs has a considerable effect on whether bacterial MAMPs will be recognized, and if so, whether their recognition promotes pro-inflammatory responses or represses them. Pathogenic bacteria possess virulence factors to allow them to attach to or invade IECs thereby introducing MAMPs into the IEC cytosol where they are recognized by NLRs; however, bacterial strains of the indigenous microbiota are non-invasive and therefore less potent activators of NLRs. In the intact epithelium,

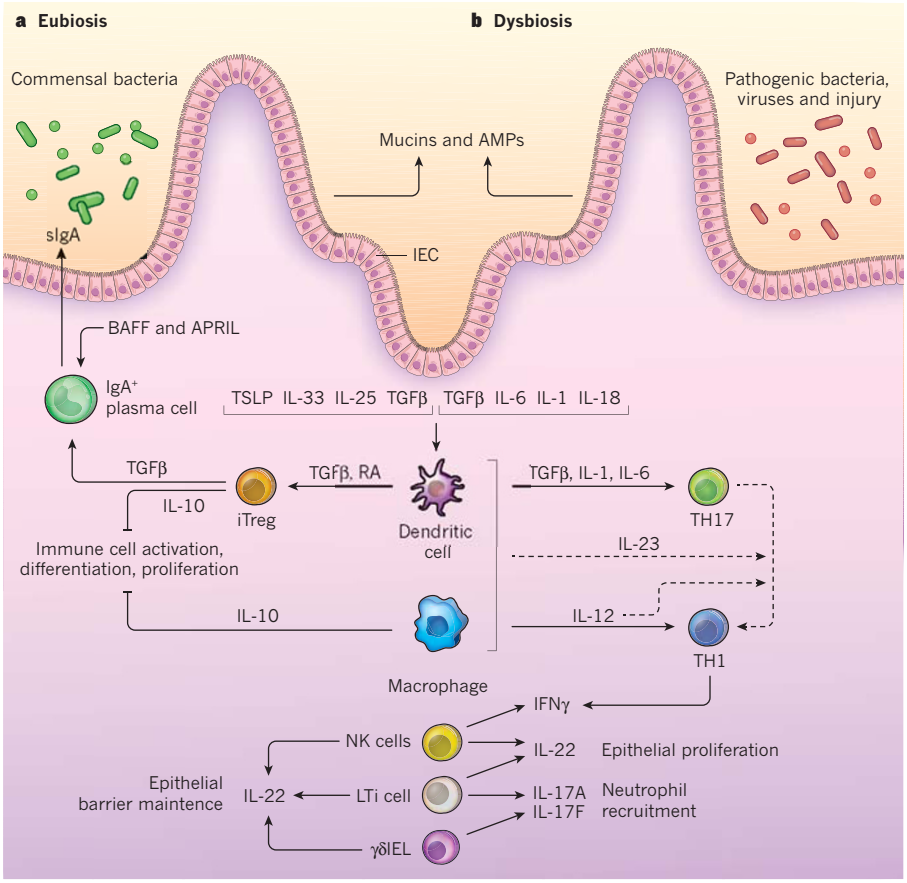

Figure 3 | The epithelial-innate-adaptive continuum in response to microbial antigens. **a**, In response to the microbiota, IECs secrete mucins and AMPs that limit microbial interaction with epithelial cells. Under homeostatic, eubiotic conditions, MAMPs stimulate the secretion of cytokines (including TSLP, IL-33, IL-25, and TGFβ) by IECs that promote development of tolerogenic macrophages and dendritic cells. Dendritic cells, in turn, induce the development of induced T_{reg} (iT_{reg}) cells through a TGFβ- and retinoic acid (RA)-dependent process. Through multiple mechanisms, including the secretion of TGFβ and IL-10 by iT_{reg} cells and the secretion of IL-10 by macrophages, the anti-inflammatory balance of the intestine is maintained by inhibiting or dampening potential effector responses. In addition, T_{reg} cell-derived TGFβ and epithelial-derived BAFF and APRIL promote development of IgA^+ plasma cells to ensure an abundant supply of sIgA in the lumen that further limits microbial interaction with the epithelium. **b**, In the face of pathogen invasion, mucosal injury or dysbiosis, MAMPs stimulate the secretion of pro-inflammatory cytokines by IECs (including, IL-6, IL-1 and IL-18) and intestinal dendritic cells and macrophages (including IL-6, IL-23 and IL-12) that induce development of the effector $CD4^+$ T cells T_H1 and T_H17, the latter of which can transition to the former as a result of IL-23 or IL-12 signalling. Intestinal innate lymphoid cells, including NK-like cells, LTi cells, and γδIELs, respond to pro-inflammatory cytokines to upregulate IL-22, which helps to protect the epithelial barrier, and IL-17A and IL-17F, which are involved in neutrophil recruitment.

MAMPs derived from commensals are largely restricted to interactions with apically accessible TLRs, which seem to be functionally dampened by tonic exposure to the microbiota, whether through decreased expression, impaired signalling or both.

By contrast, expression of TLRs on the basolateral aspects of IECs can signal a barrier breach by both commensals and pathogens. This is exemplified by TLR5, the receptor for flagellin, which is expressed on the basolateral aspects of the epithelium where it can detect the repeated flagellin monomers that make up bacterial flagellae[32]. Detection of flagellin on the 'host' side of the intestinal epithelial barrier results in epithelial-cell activation, which initiates inflammatory responses by innate and adaptive immune cells with the aim of eradicating the invading bacterium. The importance of flagellin as an immune-activating MAMP and an antigen targeted by adaptive immune cells is reflected in its high representation among antigens bound by serum antibodies from colitic mice and in patients with Crohn's disease[33].

Apical expression of TLRs and restricted access to cytosolic NLRs are adaptations to constrain interactions with luminal microbes, but some commensals have also evolved strategies that actively dampen TLR signalling in IECs, should contact occur. *Bacteroides* and *Lactobacillus* spp. inhibit activation of the classical NF-κB pathway, which is central to induction of pro-inflammatory gene expression downstream of all PRRs. Active NF-κB dimers are sequestered in the cytosol by association with IκB complexes. Release of NF-κB for nuclear localization is contingent on the phosphorylation of IκB, by which it is targeted for ubiquitylation and protesomal degradation downstream of PRR signalling. Contact of IECs with these commensals inhibits IκB degradation, thereby blocking nuclear transport of NF-κB (ref. 34). *Bacteroides* spp. can further dampen NF-κB signalling by enhancing the nuclear export of NF-κB by inducing increased expression of the nuclear receptor family member, peroxisome-proliferation-activated receptor-γ (PPAR-γ). PPAR-γ binds NF-κB in the nucleus and shuttles it back to the cytoplasm, thereby terminating its transactivation of pro-inflammatory genes[35]. Thus, in addition to host mechanisms that normally restrain direct contact between commensals and IECs, co-adaptation of the microbiota and host have provided mechanisms that suppress pro-inflammatory engagement of PRRs in the intact epithelium.

The epithelium also supports tolerance of the microbiota by conditioning intestinal dendritic-cells through directional release of immunomodulatory cytokines into the lamina propria. At homeostasis, IECs secrete thymic stromal lymphopoietin (TSLP), IL-33 and IL-25, which promote tolerogenic activities of a subset of intestinal dendritic-cells defined by expression of integrin $\alpha_E\beta_7$ complex (also known as CD103)[36]. Antigens presented by $CD103^+$ dendritic-cells favour the development of T_{reg} cells and IgA^+ plasma cells that 'home' to the lamina propria at which they repress inflammatory responses to the microbiota[37]. IECs also produce abundant TGF-β, which suppresses NF-κB-dependent pro-inflammatory signalling in intestinal macrophages and dendritic cells, and promotes development and maintenance of T_{reg} cells and IgA^+ plasma cells. TLR-activated IECs can directly promote T-independent development of sIgA producing plasma cells in ILFs by the release of both B-cell activating factor (BAFF) and a proliferation-inducing ligand (APRIL)[38]. In addition to cytokines, IECs express other factors that limit pro-inflammatory responses of mucosal immune cells, including products of tryptophan metabolism and prostaglandins (for example, PGE_2).

Like IECs, intestinal macrophages normally express lower levels of TLRs and are hyporesponsive to TLR signalling to dampen inflammatory responses to the commensal microbiota. This is despite their retention of highly active phagocytic and bactericidal activities[39]. Also like IECs, this inflammatory anergy is probably multifactorial, but reflects the limited entry of commensal bacteria or their products into the cytosol in which they can activate NLRs — despite their uptake and degradation in phagolysosomes. Although phagocytosis of commensal bacteria induces pro-IL-1β in intestinal macrophages, its cleavage by caspase-1 to produce the active form requires NLRP3- or NLRC4-containing inflammasomes, which are activated by MAMPs

that are more effectively delivered into the cytosol by pathogenic, rather than commensal, bacteria[40]. Therefore, phagocytes in the intestinal lamina propria are conditioned to mount inflammatory responses against pathogens that deliver MAMPs to cytosolic sensors, but to efficiently clear commensals in a non-inflammatory manner.

Innate lymphoid–cells response to epithelial barrier breach

Although LTi cells are indispensable in the development of the GALT, they are only one subset of a growing family of ILCs that have emerged as important contributors to intestinal homeostasis and barrier defence in the postnatal immune system[41,42]. The diversity of ILC subsets seems to parallel that of $CD4^+$ T cells, with which they share many functional features. However, the antigen-specific-receptor-lacking ILCs are recruited to mucosal immune responses mainly by cytokines, which are produced by IECs or intestinal myeloid cells (dendritic cells and macrophages).

ILCs are distributed in the intestinal lamina propria and GALT, in which they are poised to respond rapidly to microbes that penetrate the epithelial defences. LTi cells are particularly important to barrier defence against bacterial incursions[43], as are a subset of natural-killer-like cells (referred to as NK-22 or ILC22 cells), which seem to share many features with LTi cells[44]. Common to these cells, and to $CD4^+$ T cells of the T_H17 lineage (discussed later) is their expression of the transcription factor retinoic acid-related orphan receptor (ROR)γt, which is required for these cells' development and function[9], and production of the cytokines IL-22, IL-17A and IL-17F (ref. 45). IL-22 is a member of the IL-10 cytokine family that acts on epithelial cells of barrier tissues, including the intestine, to enhance antimicrobial defence and epithelial barrier integrity. IL-17A and IL-17F target innate immune cells, stromal cells and epithelial cells to induce cytokines and chemokines (for example, G-CSF and CXCL8) that induce increased neutrophil production and recruitment, respectively.

$ROR\gamma t^+$ ILCs seem to be the main source of IL-22 at steady state and, in a continuous feedback loop with the microbiota, release IL-22, IL-17 or both in response to microbiota-induced production of IL-23 by macrophages and dendritic cells. These cytokines stimulate epithelial -cell secretion of antimicrobial peptides that inhibit or kill bacteria in the vicinity of the epithelial cell surface. Although ILCs can contribute to intestinal pathology under certain circumstances[46], they are crucial in the early host response to enteropathogenic bacteria[41,47].

$ROR\gamma t^+$ ILCs also express the aryl hydrocarbon receptor (AhR) with which they sense microbiota metabolites and xenobiotics[45,48]. AhR signalling is required both for ILC maintenance and IL-22 production[48]. Akin to its essential role in GALT development, ILC production of lymphotoxin provides an important amplifying loop for the production of IL-22 by ILCs, stimulating dendritic cell production of IL-23 that, in turn, increases IL-22 production by ILCs[49,50]. In addition to RORγt and AhR, another transcription factor shared between innate and adaptive immune cells that influences the composition of the microbiota is T-bet. This transcription factor is involved in T_H1 cell development, and is also expressed by a distinct subset of ILCs. Under pathogen-free conditions, RAG-deficient mice, which lack B and T cells, are relatively resistant to inflammation driven by their microbiota, despite the absence of an adaptive immune system. This is largely owing to compensatory increases in ILCs. However, when deficient for T-bet, RAG-deficient mice develop spontaneous intestinal inflammation resembling ulcerative colitis (known as T-$bet^{-/-}$ $RAG^{-/-}$ ulcerative colitis (TRUC) mice)[51]. Disease is a result of overexpression of TNFα by dendritic cells and an absence of T_{reg} cells. Remarkably, transfer of the microbiota from TRUC mice to immunocompetent mice transfers disease, reflecting the outgrowth of two pathogenic bacterial strains: *Klebsiella pneumoniae* and *Proteus mirabilis*. Although details of the mechanisms responsible for T-bet-dependent restraint of the microbiota remain to be defined, the dysbiosis in TRUC mice highlights the importance of innate immune cells in regulating the composition of the microbiota and is evidence that a dysbiotic flora can drive disease in otherwise healthy hosts.

Adaptive immunity in homeostatic responses to microbiota

The interplay between the microbiota, intestinal epithelium and innate and adaptive immune cells at homeostasis favours the dominance of regulatory networks that prevent inflammation or immune-mediated disease. Remarkably, the innate defences are sufficiently robust that much of the host response to the microbiota progresses without involvement of $CD4^+$ T-cell-dependent responses. Furthermore, a portion of the sIgA response that contributes to partitioning of commensal organisms away from the epithelium is generated in ILFs without the requirement for T cells[13]. When the adaptive immune response is recruited, it is typically limited to the mucosal tissues such that systemic immunity is not generated. This compartmentalization of mucosal immunity is a result of the programming of adaptive immune cells to travel back to the mucosae following their differentiation in Peyer's patches or MLNs. Finally, at steady state, antigen presentation by intestinal dendritic cells favours the development of regulatory $CD4^+$ T cells, which suppress the development of pro-inflammatory innate and effector T-cell responses to avert excessive inflammation.

Regulatory T–cell responses to the microbiota

$CD4^+$ T_{reg} cells are essential for maintenance of mutualism with the microbiota. The frequencies of these T cells are considerably elevated in the intestine relative to other tissues[52], and microbiota-induced T_{reg} cells affect the composition of the microbiota[53]. The crucial role of T_{reg} cells in immune homeostasis to the microbiota is well-documented by the consequences of these cell's absence[54]. T_{reg}-cell deficiency, irrespective of the means by which it is induced, results in unopposed effector T-cell responses and IBD, driven by reactivity to antigens of the microbiota. Indeed, much of our understanding of the inflammatory potential of the commensal microbiota has been garnered through models in which regulatory pathways have been disrupted[54]. The largely non-overlapping TCR specificities of regulatory and effector T-cell subsets in the colonic lamina propria and associated lymphoid tissues suggest that distinct antigenic epitopes, whether derived from the same or distinct members of the microbiota, control these competing T-cell fates[55].

Regulatory $CD4^+$ T cells include subsets that are distinguished on the basis of their expression of the transcription factor, Foxp3. $Foxp3^+$ (T_{reg}) or Foxp3- (T regulatory cells 1 (Tr1)) are each characterized by their production of IL-10, one of the main immunoregulatory cytokines required for immune tolerance of the intestinal microbiota. The non-redundant role of IL-10 in intestinal immune homeostasis is well-established; IL-10-deficient mice develop spontaneous, unremitting colonic inflammation driven by IL-23 and the T_H17 pathway[56,57]. The essential role of TLR in sensing the microbiota in this process is evident by the absence of disease in germ-free IL-10-deficient mice and mice deficient for both IL-10 and MyD88 (ref. 58, 59). Although non-T-cells produce IL-10, T-cell-derived IL-10 is crucial for intestinal immune homeostasis. Thus, IL-10 deficiency that is restricted to total $CD4^+$ T cells results in spontaneous colitis that has a severity comparable with that in mice with global loss of IL-10 (ref. 60). Although deficiency of IL-10 that is limited to $Foxp3^+$ T_{reg} cells also induces colitis, its severity is reduced, indicating that $Foxp3^-$ $CD4^+$ T cells contribute to the protective IL-10 response.

Myeloid cells of the innate immune system are the main targets for regulatory actions of IL-10 (ref. 61), although IL-10 signalling in $CD4^+$ T cells also seems to be contributory[62,63]. Importantly, polymorphisms in the IL-10 locus confer susceptibility to IBD in humans[64], and early-onset IBD has been linked to mutations in IL-10 receptor components that impair signalling[65].

Remarkably, IL-10-producing T cells can be induced to develop in response to specific commensals or their products. The capsular polysaccharide-A moiety of the common commensal *Bacteroides fragilis* mediates interaction of *B. fragilis* with the colonic mucosa, facilitating both colonization and activation of an anti-inflammatory cascade[66]. Polysaccharide-A promotes expansion of colonic IL-10-expressing $Foxp3^+$ T_{reg} cells, through the TLR2–MyD88 pathway[66]. The

expression of TLR2 on CD4 T-cells suggests there is the capacity for direct regulation by microbial products that favours polysaccharide-A-dependent T_{reg}-cell responses over T_H1 responses[67], although there are other known TLR2 ligands that do not promote induction of Foxp3 or IL-10 (ref. 66).

A cocktail of Clostridium species, mostly comprised of clusters IV and XIVa, drives the efficient expansion of T_{reg} cells in the colons of germ-free mice, seemingly owing to their superior ability to elicit TGFβ production in the intestinal mucosa[68]. In contrast with induction of T_{reg} cells by *B. fragilis*, *Clostridium*-dependent T_{reg} expansion occurs independently of MyD88 through mechanisms yet to be defined[68]. To date, there are no studies using otherwise complete microbiota that are devoid of *B. fragilis* or this specific collection of Clostridium spp., leaving open the issue of the level of redundancy in this process. However, *B. fragilis* is absent from the microbiota of 20–30% of humans, indicating the existence of other pathways to T_{reg}-cell development. Accordingly, default induction or expansion of T_{reg} cells can be achieved by colonization of germ-free mice with a defined altered Schaedler flora (ASF) that consists of eight species of the microbiota dominated by *Bacteroides distasonis*, but devoid of *B. fragilis*[69]. Thus, although there seems to be a proclivity of certain components of the microbiota to promote T_{reg}-cell development, this does not seem to be a unique property of any one microbial component, reflecting substantial redundancy in the system to ensure that T_{reg}-cell dominance is established.

Effector T-cell responses to the microbiota

Similar to T_{reg} cells and B cells, the largest deployment of effector $CD4^+$ T-cells in the healthy immune system is in the intestines, and is largely driven by the microbiota. Although the intestines mount robust T_H2 responses to helminth infestations, in countries where these infections are less common the main effector T-cell subsets resident in the gut express cytokines characteristic of T_H17 and T_H1 cells.

T_H17 cells in particular, which are thought to be the most ancient of effector T-cell subsets[4], seem to have evolved to bolster mucosal barrier defences to promote mutualism with the microbiota. T_H17 cells share developmental requirements for RORγt and AhR (ref. 70) with intestinal ILCs, as well as a similar effector cytokine repertoire, including IL-17A, IL-17F and IL-22. Furthermore, T_H17 cells have developmental ties with T_{reg} cells through their mutual requirement for the abundant intestinal cytokine TGFβ (ref. 4). Finally, the T_H17 developmental pathway is distinguished by considerable plasticity[71], allowing divergent functional programs contingent on local pro- or anti-inflammatory cues. Although a last line of defence, effector $CD4^+$ T-cells are perpetually engaged in the host–microbiota dialogue by favouring regulatory benefits at homeostasis or pathological consequences when dysregulated.

The contribution of the microbiota to intestinal T_H17-cell development is highlighted by the virtual absence of this subset in germ-free mice[72]. In addition to IL-6, which is required for T_H17 differentiation and its deviation away from induced T_{reg} programming, multiple microbiota-dependent factors favour T_H17 development in the intestines, including TGFβ, IL-1β (ref. 73), IL-23 (ref. 74) and even ATP derived from commensal bacteria[75]. Similar to the ability of a limited quorum of commensal bacterial species to disproportionately influence induced T_{reg}-cell development, minor constituents of the microbiota can amplify intestinal T_H17 cell numbers. In mice, an unusually potent, but not unique, inducer of T_H17 cells[69] is the *Clostridia* sp. *Candidatus arthromitus* or segmented filamentous bacteria (SFB)[72,76]. This bacterium populates the ileum and caecum and has long been known to be a potent activator of intestinal immune responses[77]. Induction of T_H17 cells by SFB provides protection against gut pathogens[78], suggesting that general amplification of T_H17 effector cells can be host-protective. However, T_H17 induction by SFB is not entirely benign, because mono-association of mice with SFB induces T_H17-mediated inflammatory arthritis[79] and multiple-sclerosis-like symptoms in the experimental autoimmune encephalomyelitis (EAE) model[80]. Remarkably, therefore, sensing of a single constituent of the intestinal microbiota can promote autoimmunity in extraintestinal tissues. At present, it is unclear whether SFB, or related organisms, exert similar effects in humans.

Whether at steady state, responding to infection or chronically inflamed as a result of IBD, the intestine contains, in addition to effectors that express IL-17, T cells that express IFNγ or both IL-17 and IFNγ. Of note, T_H17 cells retain a high capacity for divergent cytokine expression profiles and function, or plasticity, after their commitment to the T_H17 pathway. Specifically, T_H17 cells can give rise to IFNγ producers that resemble classical T_H1 cells. This has been demonstrated in humans and mice[71,81], and has suggested that many of the T_H1 cells found in the intestine arise from the T_H17 pathway (Fig. 3). The relative contributions of T_H1 cells to immune protection or pathogenesis remain to be elucidated, whether they are derived from the T_H17 pathway or not.

Balancing regulatory and effector responses

To balance accommodation of the microbiota and the need to mount host defences against microbial invasion, the adaptive immune system has evolved to direct the development of distinct $CD4^+$ T-cell fates by different APC subsets and the microbe-induced factors they produce. Although T_{reg}-cell dominance is the default at homeostasis, the shared dependence of induced T_{reg} and T_H17-cell development on TGFβ provides an elegant means to alternately divert programming of naive $CD4^+$ T cells from a homeostatic, microbe-tolerant response to an inflammatory, microbe-clearing response contingent on the cofactors integrated with TGFβ signalling[4] (Fig. 3).

At homeostasis, naive $CD4^+$ T-cell recognition of antigens derived from the microbiota favours induced T_{reg}-cell development by virtue of the production, by intestinal dendritic cells, of the vitamin A metabolite all-trans-retinoic acid[82–84]. Vitamin A is not synthesized by the host so its influence on the regulatory tone of the mucosal immune response is predicated from adequate dietary intake. Retinoic acid is derived from vitamin A by the sequential actions of the ubiquitous enzyme, alcohol dehydrogenase and one of three retinal-specific aldehyde dehydrogenases, which are more restricted in their tissue distribution, including $CD103^+$ dendritic cells and IECs in the intestine. The potency of retinoic acid as a cofactor for TGFβ-dependent development of induced T_{reg} cells — and suppression of T_H17 development — and its constitutive production by a regulatory subset of intestinal dendritic cells at steady state reflect the robust default pathway to T-cell-mediated regulation. Under conditions in which microbial antigens promote production of pro-inflammatory cytokines by dendritic cells, retinoic acid is either repressed[85] or co-opted by pro-inflammatory pathways[86,87] to override T_{reg}-cell induction.

In common with T_H17 cells, and perhaps reflecting an aspect of shared TGFβ-dependent programming that favours multipotency, induced T_{reg} cells display developmental plasticity that influences adaptive immunity to the microbiota. When acted on by pro-inflammatory cytokines, induced T_{reg} cells can down-regulate Foxp3 and up-regulate RORγt (ref. 88), and T_{reg} cells can be converted into IL-17- or IFNγ-expressing effectors during infection[89]. Notably, although $Foxp3^+$ T_{reg} cells may be diverted to effector responses, the reverse is less apparent; established effector T cells, including T_H17 cells, seem to be more resistant to reprogramming to become $Foxp3^+$ regulatory cells. Accordingly, microbial antigens that are identified as products of a pathogen are likely to be remembered as such through their imprinting of an effector T-cell response. By contrast, microbial antigens that promote induced T_{reg} responses at homeostasis, may, in the context of an inflammatory response, reprogram induced T_{reg} cells to generate an effector T-cell response that breaks the 'tolerance' to that microbe.

In a further example of the developmental plasticity of T_{reg} cells, recent studies report their role in IgA class-switch recombination in the gut, representing another arm of T_{reg} cell function in promoting immune homeostasis to the microbiota. TGFβ has long been recognized as the principal switch factor for development of IgA-producing B cells. In a new twist on TGFβ's role in this process, induced T_{reg} cells were found to down-modulate Foxp3 and acquire features of follicular helper T cells

that promoted IgA production in Peyer's patches[90]. Depletion of T_{reg} cells also caused a rapid loss of IgA^+ plasma cells and sIgA production in the intestine[91], indicating that T_{reg} cells participate both in the development and maintenance of intestinal IgA^+ B cells. Although known to be important in host defence against infections, the main role of sIgA is to establish mutualism with the intestinal microbiota, as evidenced by its marked depletion in germ-free mice and systemic IgG response to the microbiota in animals that specifically lack sIgA. Coupled with the fact that about 75% of sIgA reactive to the microbiota develops through the T-cell-dependent pathway[91,92], this suggests that sIgA is mainly regulated by intestinal T_{reg} cells. In addition to providing evidence of further plasticity in the T_{reg} developmental program, these findings identify an additional link between adaptive immune networks that cope with the commensal flora, and extend the long-established role of TGFβ in promoting host-microbiota mutualism[4].

Dysregulated immunity to the microbiota

Owing to their defined antigenic specificity and durable memory, effector $CD4^+$ T cells are a liability when inappropriately directed against self-antigens or, in the case of the microbiota, the 'extended' self. In view of the enormous number of antigens expressed in the intestinal metagenome, it is remarkable that dysregulated effector responses to the microbiota are the exception. When they do occur, the result is IBD, including Crohn's disease or ulcerative colitis, which have distinct clinical and histopathological features, but a common requirement for the intestinal microbiota. Similar to autoimmunity directed against host self-antigens, dysregulated $CD4^+$ T-cell responses to antigens of the microbiota leads to chronic, typically relapsing and remitting disease that reflects the immune system's inability to eliminate the antigens that drive the abnormal response.

That being said, inflammatory immune responses in the intestines result in detectable alterations of the resident microbiota, irrespective of the cause[93]. This presents a challenge in our attempts to understand how certain microbes positively or negatively affect the disease process in IBD because it is difficult to draw conclusions without knowing the composition of the microbiota before diagnosis. As such, our understanding of the antigenic components of the microbiota that are the targets of immune attack in IBD are limited. However, experimental mouse models and patients with Crohn's disease show a remarkably shared reactivity to specific flagellin epitopes expressed by *Clostridium* spp., which seem to be immunodominant despite their minor representation within the commensal flora[33,94]. What attributes of these commensals make them particularly common targets in a large subset of patients with Crohn's disease — but not ulcerative colitis — are unknown. However, it is likely to reflect unique properties that place them in specific geographical niches in the microbiota or in intimate contact with the immune system where their expression of flagella, which is less typical for commensal bacteria, make them unusually proficient at inducing effector rather than regulatory responses. Irrespective of the basis, retention of these organisms in the collective despite the pathogenic risk they pose implies there is a benefit to host–microbiota mutualism that has been evolutionarily conserved and remains to be understood.

In view of the prominent role of the T_H17 pathway in adaptive immunity to the intestinal microbiota and propensity for generating inflammation promoted by both IL-17 and IFNγ, it is not surprising that this pathway has emerged as a major contributor to IBD pathogenesis. Prior to the discovery of T_H17, Crohn's disease and ulcerative colitis were viewed in the context of T_H1- and T_H2-centric mechanisms, respectively; but, increasingly, data from genome-wide association studies implicate the contributions of the T_H17 pathway in both diseases[95]. The discovery of IL-23 revolutionized views on the immunopathogenesis of autoimmunity and led to discovery of T_H17 cells[96]. It is therefore fitting that this was the first cytokine linked to IBD by genome-wide association studies; variants of its receptor (IL-23R) have been found to confer both protection and susceptibility to Crohn's disease and ulcerative colitis[95].

As the number of genome-wide association and next-generation sequencing studies has proliferated, so has the number of genes linked to the T_H17 pathway in IBD (ref. 95). This has been supplemented by genes of innate and adaptive immune pathways that are integrated with the T_H17 response and control it, spanning the gamut from epithelial barrier integrity maintenance and restitution to microbial sensing, to immunomodulatory cytokines. Because individual susceptibility alleles typically confer minor risk, multiple genetic susceptibility alleles are generally required for disease. Efforts to define this clustering of risk alleles is increasingly defining genotypes that confer substantial risk, as well as identifying interlaced susceptibility pathways that identify immunopathogenetic subsets of IBD — including those that do not directly implicate the T_H17 pathway. However, as more IBD susceptibility genotypes are found to overlap with extraintestinal immune-mediated diseases that do share a T_H17 pathogenesis, they increasingly implicate a role for the microbiota in conferring disease risk beyond the confines of the gut. As we advance into the era of integrated metagenomics of the human–microbiota 'superorganism', the opportunities for personalized medicine that were originally envisioned from sequencing of the human genome may well multiply in proportion to the increase in genes contributed by our microbiota, with attendant opportunities for more specifically targeted treatments and prevention of diseases that have their origins in the merger of vertebrates and their microbiomes millions of years ago. ■

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